Attorney's Docket No. 5470-238 PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re: MacDonald et al.
Serial No. 09/288,837 Group Art Unit: 1648
Filed: 8 April 1999 Examiner: Z. Lucas
For: *METHODS AND MODIFIED CELLS FOR THE TREATMENT OF CANCER*

Commissioner for Patents
Washington, DC 20231

Supplemental Declaration of Robert A. Olmsted, Ph.D.
Pursuant to 37 C.F.R. § 1.132

I, Robert A. Olmsted, do hereby declare and state as follows:

1. I am Vice President for Research at AlphaVax, Inc., the exclusive licensee of U.S. Application Serial Number 09/288,837 (*hereinafter*, "the '837 application").

2. I commissioned a study on the use of alphavirus replicon particles expressing a cancer antigen to evaluate whether vaccination with alphavirus replicon particles encoding the rat *neu* antigen (P185) provides protection to transgenic mice that contain the rat *neu* gene and spontaneously develop mammary tumors.

3. Mice transgenic for the rat *neu* gene under the control of the mouse mammary tumor virus (MMTV) promoter (known as OncoMouse®) were obtained from Charles River Laboratories. These animals spontaneously develop lethal *neu*$^+$ mammary tumors between 100-150 days post-birth. These animals express the rat *neu* transgene during embryonic development, and therefore this tumor antigen is regarded by the mouse as a "self" antigen. This transgenic mouse is considered a state-of-the-art model in the tumor immunology field.

4. My laboratory produced Venezuelan Equine Encephalitis (VEE) replicon particles (hereinafter "VRP") encoding the rat *neu* cancer antigen, generally according to the methods described in Example 1 of the '837 application, using two helper vectors encoding the VEE 3014 structural proteins. The full-length rat *neu* oncogene (GeneBank accession #X03362; Bargmann et al., (1986) *Nature* 319: 226-230) was obtained from the plasmid pSV-*neu* and cloned into the VRP.

5. Animal studies were performed at an academic research institution using these VRP. Groups of 10 mice were vaccinated subcutaneously in the

footpad with 1 x 10^6 VRP-*neu* or an equivalent dose of a control VRP encoding influenza HA antigen (VRP-HA) on days 50, 70 and 90 post-birth.

6. All 10 mice vaccinated with the control VRP-HA developed tumors in most mammary glands between days 125 to 200 post-birth, and all were sacrificed due to morbidity. In marked contrast, vaccination with VRP-*neu* provided complete protection from tumor formation. Clinical tumor development was not detected in any of the VRP-*neu* treated mice. Mice were followed to 250 days post-birth, during which time all mice remained free of detectable tumors.

7. These results demonstrated that vaccination with VRP-*neu* was able to break tolerance to a self-antigen in an animal model for breast cancer that is considered one of the most relevant models currently available in the art.

8. I do hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 19 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Robert A. Olmsted, Ph.D.

March 10, 2003
Date